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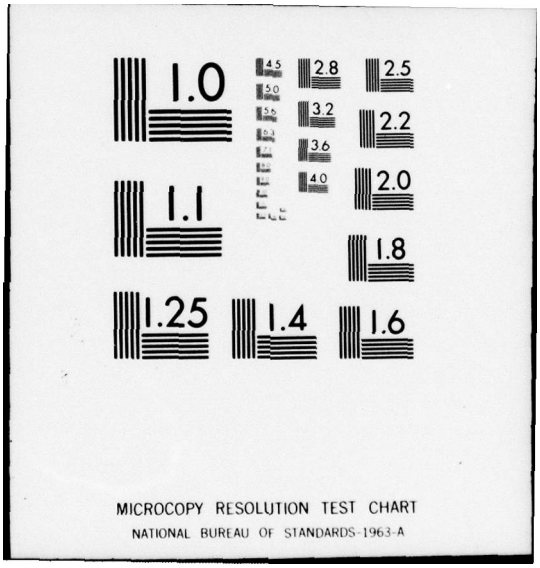
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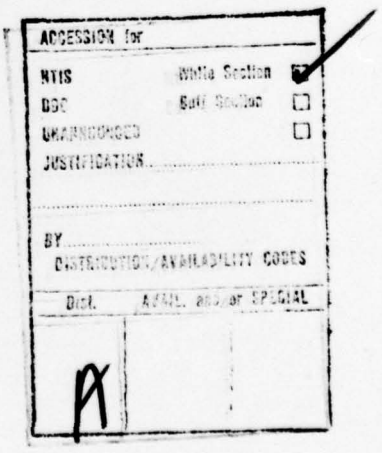
ABSTRACT
Line 20 - DD 1473

This Contract No. DAAG29-76-C-0005 helped to maintain the collection of mutant, inbred, and synthetic stocks in all available species of the flour beetles of the genus Tribolium, particularly Tribolium castaneum and T. confusum. Ancillary efforts have been made to determine the mode of inheritance and allelism of several mutations found in "natural" populations of T. confusum in Yugoslavia; and linkage relationships of "extra large" mutant found in Georgia; in the same species. The map position of the genes "black", "light ocular diaphragm aureate" has been determined in T. castaneum. Sex differences in recombination, attributed to modifiers, have been observed in linkage group III of this flour beetle. A copy of the manuscript is attached.



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Contract #DAAG29-76-C-0005

"MAINTENANCE OF TRIBOLIUM STOCK CENTER"

FINAL TECHNICAL REPORT

Dr. Alexander Sokoloff
Principal Investigator
Professor of Biology

January 26, 1977

U. S. ARMY RESEARCH OFFICE

CONTRACT # DAAG29-76-C-0005

INSTITUTION

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FINAL REPORT

Contract No. DAAG29-76-C-0005, completed on 30 November 1976, was awarded to the Foundation of the California State College, San Bernardino, Dr. Alexander Sokoloff, Principal Investigator, to maintain the collection of wild type, synthetic, inbred and mutant strains of flour beetles in the genus *Tribolium* in the Tribolium Stock Center, Department of Biology, California State College, San Bernardino, California, 92407. This collection, without parallel anywhere else in the world, constitutes an important biological research resource.

With the help of graduate and undergraduate students we were able to:
1. Continue the maintenance of most of the wild type, synthetic, and mutant stocks at regular intervals.
2. Maintain the inbred stocks. (Some of these stocks have been maintained for over 110 generations of brother-sister mating and it would take more than 10 years of effort to duplicate.)
3. Eliminate some of the less useful mutant stocks, i.e. those exhibiting either poor penetrance or extremely low viability, and which, therefore, require intensive care for their maintenance.
4. Determine the mode of inheritance and allelism of several mutations found in "natural" populations of *T. confusum* in Yugoslavia. One of the mutations producing a black body color is allelic to the McGill black mutant. Another is a sex-linked recessive modifying the eye color from black to red. Allelism tests to the previously found "eyespot" and "red" mutations have not yet been carried out, but we will do so as soon as additional help is available. The third, fas-3, is an autosomal mutant modifying the antennae to produce fusions of several antennal segments. Tests of allelism and linkage tests with two previously found mutations designated fas-1 and fas-2 have yet to be carried out.
5. Set up the initial crosses to determine the linkage relationships of the mutation "extra large" with known genetic markers.
6. Complete the determination of the map position of the genes black (b), light ocular diaphragm (lod), and aureate (au) in *T. castaneum*. Furthermore, supporting evidence was obtained to show the distance between various gene varies, depending on the cross: b++/+lod au ♀ x +lod au/+lod au crosses give the following recombinations values: au-lod = 18.32 ± 1.21%; b-lod = 21.05 ± 1.51% and b = au = 37.43 ± 1.27%. The reciprocal crosses give au-lod = 27.67 ± 1.62%; b = lod = 13.97 ± 1.26% and b-au = 39.79 ± 1.78%. For the larger distances encompassed in the b-au region the recombination values in the two sexes were not significantly different. For the shorter b-lod region the recombination values were significantly greater in the females than in the males, while for the adjacent lod-au region the opposite was true. On the basis of the current literature it appears that the main factors contributing to these sex-differences in recombination are the modifiers which are different in the genetic background of the two sexes. A short paper dealing with this topic has been submitted to the Canadian Journal of Genetics and Cytology, and it will appear in the March, 1977 issue. A copy of the manuscript is attached.

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Mr. Daryl Faustini, a graduate student, has shown in his Master of Science thesis that irradiation of *au lod* beetles at the level of 4000 rads significantly increases recombination frequency in males but not in females.

7. Because of considerable changes in the holdings of stocks in the Tribolium Stock Center we took some time to revise our stock lists. The revised stock list will appear in the Tribolium Information Bulletin (19 and 20).
8. In the year 1976 we received for deposit 18 stocks, and distributed to colleagues within the United States and abroad 53 mutant and wild type stocks

The activities of the Tribolium Stock Center will be supported for the next two (and possibly three) years through a research grant: DRXRO-CB-14447-L from the U.S. Army Research Office.

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SEX AND CROSSING OVER IN LINKAGE GROUP III OF *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA, TENEBRIONIDAE)

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Contract #DAAG29-76-C-0005

ABSTRACT

The relative position of the genes black (b), light ocular diaphragm (lod) and aureate (au) for the third linkage group of T. castaneum has been determined as b - lod - au. The distances between the various genes varies, depending on the cross. The b++/+ lod au ♀ x + lod au/+ lod au crosses give the following recombination values: au - lod = 18.32 ± 1.21%; b - lod = 21.05 ± 1.51% and b - au = 37.43 ± 1.27%. The reciprocal crosses give au - lod = 27.67 ± 1.62%; b - lod = 13.97 ± 1.26% and b - au = 39.79 ± 1.78%. For the larger distances encompassed in the b - au region the recombination values in the two sexes were not significantly different. For the shorter b - lod region the recombination values were significantly larger in the females than in the males, while for the adjacent lod - au region the opposite was true. On the basis of the current literature it would appear that the main factors contributing to these sex differences in recombination are the modifiers which are different in the genetic background of the two sexes.

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INTRODUCTION

In the Coleoptera, the greatest number of mutants for any one species available are some 125 spontaneous mutations which have been discovered in the tenebrionid flour beetle Tribolium castaneum, a major pest of stored products.

With the aid of these mutants ten linkage groups have been identified, corresponding to the 10 pairs of chromosomes in this species (Sokoloff 1966, 1975). However, it is by no means certain whether some of these linkage groups may not in fact represent either genes on the same chromosome arm located 50 or more units apart, or genes placed far apart on different chromosome arms. Furthermore, the chromosomes of Tribolium are too small to be useful in cytogenetic studies.

In the course of investigations to produce the available chromosome maps it has been established that
(1) recombination values for genes in coupling or in repulsion within a sex may be approximately the same (Englert and Bell 1963) or they may be different (Englert and Bell 1963) Dewees 1967, Englert 1973).
(2) recombination values for a given region in a linkage group may differ only slightly or considerably in the two sexes. (Sokoloff 1964)

Speculation has arisen to account for the differences in recombination in the two sexes. It is possible that the best explanation for the phenomenon lies in a difference in distribution of a single chiasma in the two sexes (Sokoloff 1964). However, it has not been

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possible to identify any of the linkage groups with any chromosome (only the X and Y chromosmmes can be identified readily by cytological techniques).

Be that as it may, the differences in crossover values in the two sexes are real, and present a problem to the geneticist undertaking work with beetles. Relatively recently Dawson (1972) working with markers of linkage group IV of T. castaneum showed that if genes are far apart (25-30 units) the recombination values in the two sexes are not significantly different (as Sokoloff 1964 had shown for genes in that group). However, when this region is subdivided into smaller regions, crossover values were higher in males for one region and in females for another region.

The purpose of this note is to show that the phenomenon observed by Dawson may be a general one, since it can be shown in yet another linkage group (linkage group III) in Tribolium castaneum.

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MATERIALS AND METHODS

The mutants used in this investigation were black, light ocular diaphragm, and aureate. The mutant black (b) is a semi-dominant which affects body color; the normal body color in Tribolium castaneum as the name of the species indicates, is chestnut; b/b produces a black body color, while +/b produces an intermediate color referred to as bronze (Sokoloff et al., 1960). The recessive mutant light ocular diaphragm (lod) blocks the synthesis of melanin in the ocular diaphragm, an andoskeletal structure which helps to support the ommatidia of the compound eye. The presence of a normally pigmented ocular diaphragm in eye color mutants such as pearl, red, or chestnut gives the beetles a "spectacled" appearance, since the marginal ommatidia lie over the ocular diaphragm and this structure is visible through the ommatidia. Beetles homozygotes for eye color mutant genes and lod appear non-spectacled, i.e. the ommatidia of the whole compound eye are either uniformly colorless as in the case of pearl, or they may be uniformly colored (if the mutant gene produces a color pigment in the ommatidia).

The lod mutation also can be identified in otherwise normal beetles: the compound eye in non-lod (normal) beetles is uniformly black all the way to the outer edge of the eye by virtue of both the black pigment deposited in the ommatidia and the black pigment deposited in the ocular diaphragm. In lod/lod beetles with normal eye color genes the ommatidia in the center of the eye will appear black, but the marginal ommatidia, over the ocular diaphragm, appear crystalline

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and of the body color of the beetle. A previous note placed lod 24 units away from b (Sokoloff, 1964; Sokoloff et al., 1967).

The recessive mutation aureate (au), increases the number of setae all over the body of T. castaneum. By actual count the relative number of setae in au is increased two- to threefold over the normal (Sokoloff et al., 1967).

The map position of the au, b and lod genes was determined by crossing black pearl (b, p) males with aureate, light ocular diaphragm and pearl (au lod; p) females. The pearl mutation, a marker of linkage group II, was included in these crosses to facilitate the identification of the lod and non-lod beetles in later generations. The F_1 were bronze and pearl eyed, but they showed a normal distribution of setae, and a pigmented ocular diaphragm. These F_1 beetles were isolated according to sex in the pupal stage. When the adults emerged they were crossed with au lod p beetles and introduced as single pairs into rearing jars. The rearing jars contained about 4g of standard medium, i.e. 19 parts by weight unbleached whole wheat flour, and 1 part brewer's yeast. The beetles were reared in a walk-in incubator maintained at about 29°C and 70% RH.

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RESULTS

Pearl (p), a marker gene for linkage group II, was used only to facilitate the identification of the lod phenotype and thus, will be omitted from discussion of the results. The distribution of genotypes in Table I clearly indicates linkage of all three genes, and recombination values between the three loci are:

$$b - au = 37.43\% \pm 1.27$$

$$b - lod = 21.05 \pm 1.51$$

$$au - lod = 18.32 \pm 1.21$$

for females;

$$b - au = 39.79 \pm 1.78$$

$$b - lod = 13.97 \pm 1.26$$

$$au - lod = 27.67 \pm 1.62$$

for males.

The coefficient of coincidence in the first set of data was 25.27 and in the second set of data 25.22, suggesting a moderate degree of interference of crossing over between the b - lod and lod - au regions.

The relative position of the three genes, b - lod - au, is the same in the two types of backcrosses. Furthermore, the X^2 test for homogeneity indicates that the recombination values from the two types of backcrosses for the b - au region are not significantly different (recombination values of $37.43 \pm 1.27\%$ for female and $39.79 \pm 1.78\%$ for male heterozygotes).

However, the X^2 test for homogeneity indicates that recombination values for the two sexes are significantly different for the shorter b - lod ($X^2 = 148.2$, df = 1 $P << .01$) and lod - au regions ($X^2 = 219.9$, df = 1, $P << .01$). Furthermore, it is noteworthy that the recombination

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values in the b - lod region are greater(21.05 ± 1.51%) in the female than in the male (13.97 ± 1.26%) heterozygotes, while in the lod - au region the recombination values in the female heterozygotes (18.32 ± 1.21%) are smaller than in male (27.67 ± 1.62%) heterozygotes.

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DISCUSSION

The phenomenon which brings about a difference in recombination in the two sexes is apparently widespread. Dunn and Bennett (1967) have surveyed the literature on the subject and have concluded that this phenomenon is present both in plants and animals. Their survey also showed that crossing over occurs in both sexes in the majority of animals. There is some tendency for crossover values in females to exceed those in males. They found that in the house mouse marked sex-differences can occur in opposite directions in different chromosomes.

The studies carried out on the subject of recombination in Tribolium have provided the following results:

Sokoloff (1964) found that recombination between any given pair of genes in linkage group VII of T. castaneum, whether in coupling or in repulsion, is not significantly different within a sex but it is significantly different between the sexes. The recombination values may differ only slightly or considerably between the sexes, to the point that in the male the values may approach 50%, giving the erroneous impression that the genes are not linked, while the values obtained from females clearly indicate linkage between these genes. This phenomenon was not observed in data derived from linkage group IV. Sokoloff advanced the hypothesis that the difference in recombination, reflected in the crossover values in the two sexes in T. castaneum, results from a difference in the distribution of a single chiasma.

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Johnson (1966) found that recombination is increased in the male sex for linkage group VII and in the female for linkage group IV genes.

Dewees (1967) found unequal recombination values for males and females, but these were not affected by the cis or trans position of the genes within a given sex. He suggested that the unequal recombination values may result from a differential segregation of chromosomes in one sex (probably the female) with the result that the recombinant chromosomes is incorporated more often in the polar body during second-division segregation, resulting in a lower number of recombinant progeny.

Englert and Bell (1963) reported that crossing over between genes for linkage group VIII was not equal in the two sexes in the coupling or cis phase, while in the repulsion or trans phase crossing over was equal. Dewees (1967). on the other hand, found that recombination was greater in the male than in the female for genes associated with linkage group V, regardless of cis or trans arrangement.

To remove sex as the confounding factor from the linkage phase. Englert (1969a, b) investigated recombination rates of the sex-linked recessive markers pygmy (py) and red (r) in the female. The pooled data gave a recombination rate of 11% for the cis and 7.3% for the trans phase, a significant difference.

Dawson (1972) has resolved the apparent discrepancy between the results of Sokoloff (1964) and those of Johnson (1966) for recombination values of linkage group IV mentioned above. Dawson's data support Sokoloff's conclusions that for this linkage group no difference in

the two sexes are observable for genes far apart (25-30 units); however when this region is subdivided into smaller regions, crossover values were higher in males for one region and in females for another (Dawson, 1972).

Faustini (1976) found that recombination between the light ocular diaphragm (lod) and aureate (au) genes was greater in the males (the heterogametic sex) than in females (28.17 $\pm$ .88% vs 17.41 $\pm$ .82%, respectively), data which are remarkably similar to those obtained in this experiment.

Most of the investigators in the studies mentioned above offered some kind of explanation to account for the differences in recombination in the two sexes.

In the light of more current studies by Chinicci (1971 a, b) and Dewees (1975) most of those explanations are no longer valid.

Chinicci's studies in Drosophila and Dewee's studies in Tribolium (Dewees, 1975) have shown that recombination values can be modified by selection toward a higher or a lower value, and the conclusion has been made that recombination in these insects is under polygenic control. Since males and females carry different genetic modifiers, it is not surprising that different recombination values are obtained in the two sexes.

What is of interest from the data reported for flour beetles by Dawson (1972) and in the present study is that sex-differences not only can occur in opposite directions in different chromosomes, but these sex-differences can occur in opposite directions in adjacent regions of the same chromosome.

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ACKNOWLEDGMENTS

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TABLE I

Progeny resulting from crossing (a) b++/+ lod au ♀ X + lod au/ + lod au ♂ and (b) b++/+ lod au ♂X + lod/au + lod au ♀. In each cross there were 10 replicates.

	MATING SERIES	
	(a)	(b)
b++	319	240
+ lod au	313	210
b lod au	103	46
+ + +	103	53
b lod +	2	0
+ + au	8	7
+ lod +	86	111
b + au	92	92
	1026	759

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